PORCINE LEPTIN PROTEIN, ANTISENSE AND ANTIBODY

This application is a continuation-in-part of application USSN 08/692,922 filed July 31, 1996.

BACKGROUND OF THE INVENTION

1. Field of the Invention:

This invention relates to the regulation of energy intake and metabolism in growing, finishing, lactating or nonlactating, and gestating swine. More specifically, it relates to a specific porcine polypeptide (or protein) termed leptin which is secreted by adipocytes or other cell types and which influences energy intake and metabolism, fat deposition, and weight gain in swine. In addition, this invention relates to the nucleotide sequences encoding the porcine leptin polypeptide, the antibodies directed against the porcine leptin polypeptide, and methods to determine susceptibility to fat deposition, alter energy intake, and minimize excessive fat deposition in swine.

2. Description of the Background Art:

Obesity has been declared a public health hazard by the National Institutes of Health and has prompted the food animal industry to seek methods of limiting fat deposition in food animals. Additionally, the energetic cost of having food animals convert feed energy to fat rather than lean tissue provides considerable incentive to develop technology to facilitate the efficient production of leaner meat products and to accurately match the nutrient content of the diet to the nutrient needs of the animal. To combat these health and production problems, both prophylactic and therapeutic approaches are necessary. For prophylactic purposes, it would be useful to be able to predict and

measure the propensity or susceptibility to excessive fat deposition. For therapeutic purposes, it would be of great benefit to improve current methods of minimizing the deposition of feed energy as fat in the adipocyte. Currently, neither of these desired objectives has been achieved completely.

Proteins from genes expressed only (or predominantly) in adipose tissue and for which the level of expression can be related to fat deposition serve as prime targets for approaches directed toward prediction of fat accretion potential and the control of fat deposition. For example, a mammalian adipocyte-specific polypeptide, termed p154, was reported in USP 5,268,295 to Serrero, which is incorporated in its entirety herein by reference, as being expressed in high quantities in adipogenic cell lines after cell differentiation and is abundant in the fat pads of normal and genetically obese mice. To date, however, there have been no reports of adipocyte-specific proteins expressed at different levels in fat swine as compared with normal controls.

Leptin, the protein produced by the leptin (*ob*) gene, is possibly related to fat deposition in swine because research has shown that mutations in genetically (*ob*/*ob*) obese mice resulting in excessive fat deposition are associated with altered expression of the leptin gene. Furthermore, at least one restriction fragment length polymorphism (RFLP) has been identified and related to the fat phenotype (Zhang et al., 1994, Nature 371:425). The leptin gene is expressed specifically in the terminally differentiated adipocyte (Maffei et al., 1995, Proc. Natl. Acad. Sci. 92:6957; Leroy et al., 1996, J. Biol. Chem. 271(5):2365). Additionally, leptin is a regulator of feed intake (Pellymounter et al., 1995, Sci. 269:540; Halaas et al., 1995, Sci. 269:543; Campfield et al., 1995, Sci. 269:546).

Although the murine leptin gene has been positionally cloned and a cDNA sequence reported (Nature 371:425), neither the porcine leptin cDNA nor genomic sequence is available. Thus, the insights obtained with respect to porcine metabolism is not accessible to porcine systems.

Furthermore, the biologically active purified porcine protein (i.e., leptin) has not been obtained.

SUMMARY OF THE INVENTION

The present invention provides gene sequences, polypeptides, antibodies, and methods of using them which permit the prediction and modulation of fat deposition and regulation of feed intake (i.e. appetite) in the porcine species.

In one aspect, this invention is directed to a porcine adipocyte polypeptide (i.e., the porcine leptin protein) substantially free of other porcine polypeptides, or functional derivatives thereof. The present invention includes a porcine adipocyte polypeptide of at least about 8 amino acids of the amino acid sequence depicted in FIGS. 1A-1D (SEQ. ID NO. 1), preferably the amino acid sequence depicted in FIG. 2 (SEQ. ID NO. 2), still more preferably, the amino acid sequence depicted in FIG. 3 (SEQ. ID NO: 3 and SEQ. ID NO. 4), or functional derivatives thereof.

The present invention is also directed to a single or double stranded DNA or an RNA molecule (and their respective allelic variants) consisting essentially of a nucleotide sequence that encodes the above polypeptide, the DNA or RNA molecule being substantially free of other porcine DNA or RNA sequences or, in other words, isolated or an isolate. The DNA molecule is preferably a single or double stranded DNA molecule having a nucleotide sequence consisting essentially of at least about 20 nucleotides of the nucleotide sequence depicted in FIGS. 1A-1D (SEQ. ID NO: 1), preferably, the nucleotide sequence depicted in FIG. 2 (SEQ. ID NO: 2), still more preferably the nucleotide sequence depicted in FIG. 3 (SEQ. ID NO: 3), or a sequence complementary to the nucleotide sequences depicted in FIGS. 1A-3 (SEQ. ID NO: 1 and SEQ. ID NO. 3), or an allelic variant thereof substantially free of other porcine DNA sequences. The RNA molecule is preferably

an mRNA sequence encoding the above porcine adipocyte polypeptide, or functional derivatives thereof.

Included in the invention is a DNA molecule as described above which is cDNA or genomic DNA, preferably in the form of an expressible vehicle or plasmid.

The present invention is also directed to hosts transformed or transfected with the above DNA molecules, including a prokaryotic host, preferably a bacterium, a eukaryotic host such as a yeast cell, or a mammalian cell.

The present invention also provides a process for preparing a porcine adipocyte polypeptide or functional derivatives as described above, the process comprising the steps of: (a) culturing a host capable of expressing the polypeptide under culture conditions; (b) expressing the polypeptide; and (c) recovering the polypeptide from the culture.

Also included in the present invention is a method for detecting the presence of a nucleic acid molecule having the sequence of the DNA molecule described above, or a complementary sequence, in a nucleic acid-containing sample, the method comprising: (a) contacting the sample with an oligonucleotide probe complementary to the sequence of interest under hybridizing conditions; and (b) measuring the hybridization of the probe to the nucleic acid molecule, thereby detecting the presence of the nucleic acid molecule. The above method may additionally comprise before step (a): (c) selectively amplifying the number of copies of the nucleic acid sequence.

Another embodiment of this invention is an antibody specific for an epitope of the porcine adipocyte polypeptide, or functional derivatives thereof, either polyclonal or monoclonal. Also intended is a method for detecting the presence or measuring the quantity of the porcine adipocyte polypeptide leptin in a biological sample, comprising contacting the sample with the above antibody

and detecting the binding of the antibody to an antigen in the sample, or measuring the quantity of antibody bound.

The present invention includes methods for determining the susceptibility of swine to fat deposition which comprises removing a biological sample from a pig and measuring therein the amount of the polypeptide or mRNA coding therefor, where the amount of the polypeptide or mRNA is related to susceptibility. The present invention also includes methods for determining the susceptibility of a subject to fat deposition which comprises removing a biological sample, extracting the DNA, digesting the DNA with restriction endonucleases, probing the sample with an oligonucleotide probe, separating the resulting fragments by gel electrophoresis, and relating the number of bands (banding pattern) generated by restriction enzyme digestion to fat deposition (i.e., RFLP techniques).

Another method provided herein is for evaluating the efficacy of a drug (or other agent) directed to the regulation of fat deposition and feed intake which comprises contacting the drug being tested with an adipocyte culture *in vitro* and measuring the amount of the porcine adipocyte polypeptide or mRNA that is produced by the adipocyte, the efficacy of the drug or agent being related to changing the production of the polypeptide or mRNA.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1D depicts the nucleotide sequence of the porcine leptin gene and the amino acid translation of the porcine leptin coding sequences (SEQ. ID NO: 1 and SEQ. ID NO. 2).

FIG. 2 depicts the nucleotide sequence and the amino acid translation of the coding region of the entire porcine leptin cDNA (i.e., signal peptide and secreted protein) (SEQ. ID NO: 1 and SEQ.

ID NO. 2).

FIG. 3 depicts the nucleotide sequence and the amino acid translation of the porcine leptin cDNA corresponding to the secreted porcine leptin protein (SEQ. ID NO: 3 and SEQ. ID NO. 4).

FIG. 4 shows a comparison of the porcine leptin cDNA sequence corresponding to the entire porcine leptin protein (SEQ. ID NO. 1) with the murine (SEQ. ID NO. 6) and human (SEQ. ID NO. 5) sequences .

FIG. 5 depicts the Northern blot analysis of porcine leptin mRNA.

FIG. 6 depicts the isolation of a genomic DNA clone for porcine leptin.

FIG. 7 depicts a polyacrylamide gel electrophoresis of porcine leptin protein induction and purification in *Escherichia coli*.

FIG. 8 depicts hybridization of the porcine *ob* antisense RNA with *ob* mRNA.

FIG. 9 is a bar graph showing the lipolysis of adipocytes isolated from a pig.

FIG. 10 is a western blot of a recombinant human and porcine leptin with the polyclonal antibody to synthetic peptide based on the C-terminal sequence of porcine leptin.

FIG. 11 is a chromatograph showing a polyclonal antibody to recombinant porcine leptin immunoprecipaitates leptin from pig serum, cerebral spinal fluid (CSF) and adipose extracts.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention is directed to DNA and RNA molecules and their respective allelic variants that encode a porcine adipocyte polypeptide, termed "leptin," or a functional derivative thereof, and the porcine leptin protein itself, or a functional derivative thereof. The porcine leptin protein is useful for regulation of feed intake, energy metabolism, and fat deposition in swine. Such

objectives can be achieved by administering recombinant or purified porcine leptin, altering the expression of the porcine leptin gene or administering an antibody directed against the porcine leptin protein to achieve neutralization, depending on the desired result. The porcine leptin DNA, RNA, and protein, and their respective allelic variants and functional derivatives, and antibodies specific for the protein are used in assays to predict the potential for fat deposition in swine. These molecules can also be utilized in the development of commercially valuable technology for altering feed intake and regulating fat deposition in swine, and for matching the nutrient content of the diet to the nutrient needs of the pig.

In its first aspect, the present invention provides a porcine adipocyte polypeptide termed "leptin". The term "polypeptide" as used herein is intended to include not only the porcine leptin protein and its allelic variants (i.e. those porcine leptin proteins produced by alleles of the leptin gene and functional derivatives, but also amino acid sequences having additional components, e.g., amino acid sequences having additional components such as a sugar, as in a glycopeptide, or other modified protein structures known in the art.

The polypeptide of this invention has an amino acid sequence as depicted in FIGS. 1A-1D and 2 (SEQ. ID NO. 1 and SEQ. ID NO. 2), and preferably as depicted in FIG. 3 (SEQ. ID NO. 3 and SEQ. ID NO. 4). Also intended within the scope of the present invention is any polypeptide having at least about 8 amino acids present in the above-mentioned sequence. Sequences of this length are useful as antigens and for making immunogenic conjugates with carriers for the production of antibodies specific for various epitopes of the entire protein. Such polypeptides are also useful in screening such antibodies and in the methods of the present invention directed to detection of the leptin protein in biological samples. It is well-known in the art that polypeptides of

about 8 amino acids are useful in generation of antibodies to larger proteins of biological interest.

The polypeptide of this invention is sufficiently large to comprise an antigenically distinct determinant, or epitope, which can be used as an immunogen to produce antibodies against porcine leptin or a functional derivative thereof, and to test such antibodies. The polypeptide of this invention may also exist covalently or noncovalently bound to another molecule. For example it may be fused (i.e., a fusion protein) to one or more other polypeptides via one or more peptide bonds.

One embodiment includes the polypeptide substantially free of, i.e. isolated from other porcine polypeptides. The polypeptide of the present invention may be biochemically or immunochemically purified from cells, tissues, or a biological fluid. Alternatively, the polypeptide can be produced by recombinant means in a prokaryotic or eukaryotic host cell.

"Substantially free of other porcine polypeptides" reflects the fact that because the gene for the porcine adipocyte polypeptide of interest can be cloned, the polypeptide can be expressed in a prokaryotic or eukaryotic organism, if desired. Methods are also well known for the synthesis of polypeptides of a desired sequence on solid phase supports and their subsequent separation from the support. Alternatively, the protein can be purified (i.e. isolated) from tissue or fluids of the swine in which it naturally occurs so that it is purified away from at least 90 percent (on a weight basis), and from even at least 99 percent if desired, of other porcine polypeptides and is therefore substantially free of them. Such purification can be achieved by subjecting the tissue or fluids to standard protein purification techniques such as immunoadsorbent columns bearing monoclonal antibodies reactive against the protein. Alternatively, the purification from such tissue or fluids can be achieved by a combination of standard methods, such as ammonium sulfate precipitation, molecular sieve

chromatography, and ion exchange chromatography.

As alternatives to a native purified or recombinant porcine adipocyte polypeptide molecule, functional derivatives of the porcine adipocyte polypeptide may be used. As used herein, the term "functional derivative" refers to any "fragment", "variant", "analog", or "chemical derivative" of the porcine adipocyte polypeptide that retains at least a portion of the function of the porcine adipocyte polypeptide which permits its utility in accordance with the present invention.

A "fragment" of the porcine adipocyte polypeptide as used herein refers to any subset of the molecule, that is, a shorter polypeptide.

A "variant" of the porcine adipocyte polypeptide as used herein refers to a molecule substantially similar to either the entire polypeptide or a fragment thereof. Variant polypeptides may be conveniently prepared by direct chemical synthesis of the variant polypeptide, using methods well-known in the art. Alternatively, amino acid sequence variants of the polypeptide can be prepared by mutations in the DNA (i.e. by allelic variants of the DNA) which encodes the synthesized polypeptide (again using methods well-known in the art). Such variant polypeptides include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity. Obviously, the mutations that will be made in the DNA encoding the variant polypeptide must not alter significantly the reading frame and preferably will not create complementary regions that could produce secondary mRNA structures.

"Allelic variant" as here used means an alternative form of the SEQ ID NO: 1 gene (or nucleic acid molecule), the alternative form coding for a porcine adipocyte polypeptide, or a

functional derivative thereof, that has identical or nearly identical biological activity to the porcine adipocyte polypeptide encoded by the SEQ ID NO: 1 gene. These variants or "alleles" arise from either natural or artificially induced mutations to the gene or molecule. These mutations include differences in the overall sequence of nucleic acids in the gene due to deletions, substitutions, insertions, inversions or additions.

An "analog" of the porcine adipocyte polypeptide as used herein refers to a nonnatural molecule substantially similar in structure and biological activity to either the entire molecule or a fragment thereof.

A "chemical derivative" of the porcine adipocyte polypeptide or peptide as used herein contains additional chemical moieties not normally a part of the polypeptide. Covalent modifications are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

The polypeptide of the present invention is encoded by a nucleic acid molecule, one strand of which has the nucleotide sequence shown in FIGS. 1A-1D (SEQ. ID NO. 1), preferably as shown in FIG. 2 (SEQ. ID NO. 2), and still more preferably as shown in FIG. 3 (SEQ. ID NO. 3). The present invention is directed to a DNA sequence encoding the polypeptide, or a functional derivative thereof, substantially free of other porcine DNA sequences. Such DNA may be single stranded (i.e., sense strand, antisense strand or cDNA sequence) or double stranded. The DNA sequence should preferably have about 20 or more nucleotides to allow hybridization to another polynucleotide. In order to achieve higher specificity of hybridization, characterized by the absence of hybridization to sequences other than those encoding the polypeptide or a functional derivative thereof, a length of at

least about 50 nucleotides is preferred.

The present invention is also directed to an RNA molecule (or an allelic variant thereof) comprising a mRNA sequence encoding the polypeptide of this invention, or a functional derivative thereof, and the antisense RNA (or a fragment thereof) of the mRNA. The antisense RNA is, of course, simply the complement to the cDNA sequence (cDNA corresponds to mRNA except uracil replaces thymidine; cDNA and mRNA as "sense", so the complements of these molecules are "antisense"). Antisense RNA (or antisense "oligonucleotides") are described more fully in Molecular Biology and Biotechnology, *Antisense Oligonucleotides, Structure and Function of*, Uhlmann and Peyman, pp. 38-45 (Wiley-VCH, 1995). The antisense RNA of this invention is the complement, or a fragment, of the nucleotide sequence shown in FIGS. 1A-1D and 2 (SEQ ID NO: 1), or an allelic variant thereof. If a fragment, the antisense RNA sequence should preferably have about 20, more preferably about 50 or more, nucleotides to allow binding to a complementary region of mRNA sufficient to inhibit protein biosynthesis.

The present invention is further directed to the above DNA molecules which are functional in recombinant expression systems utilizing as hosts transfected or transformed with the vehicles and capable of expressing the polypeptide. Such hosts may be prokaryotic or eukaryotic. The DNA can be incorporated into the host organism by transformation, transduction, transfection, or a related process known in the art.

In addition to a DNA and mRNA sequence, or an allelic variant thereof, encoding the porcine adipocyte polypeptide molecule, this invention provides methods for expression of the nucleic acid sequences. Further, the genetic sequences and oligonucleotides of the invention allow the identification and cloning of additional, yet undiscovered adipocyte polypeptides having sequence

homology to the adipocyte polypeptide described herein.

The recombinant DNA molecules of the present invention can be produced through any of a variety of means, such as, for example, DNA or RNA synthesis, or more preferably, by application of recombinant DNA techniques. Techniques for synthesizing such molecules are disclosed by, for example, Wu, R., et al., Prog. Nucl. Acid. Res. Molec. Biol. 21:101-141 (1978), which is incorporated herein by reference. Procedures for constructing recombinant molecules in accordance with the above-described method are disclosed by Sambrook et al. Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), which is herein incorporated by reference.

Oligonucleotides representing a portion of the porcine adipocyte polypeptide are useful for screening for the presence of genes encoding such proteins and for the cloning of porcine adipocyte polypeptide genes. Techniques for synthesizing such oligonucleotides are disclosed by, for example, Wu, R., et al.. Prog. Nucl. Acid. Res. Molec. Biol. 21:101-141 (1978).

A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the porcine adipocyte polypeptide gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified, synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA preparation derived from cells which are capable of expressing the porcine adipocyte polypeptide gene. Single stranded oligonucleotide molecules complementary to the "most probable" porcine adipocyte polypeptide-encoding sequences can be synthesized using procedures which are well known to those of ordinary skill in the art (*See e.g.,* USP 5,268,295). Additionally, DNA synthesis may be achieved through the use of automated synthesizers. Techniques of nucleic acid hybridization are disclosed by Sambrook et al.,

supra.

In an alternative method of cloning the porcine adipocyte polypeptide gene, a library of expression vectors is prepared by cloning DNA or, more preferably, cDNA (from a cell capable of expressing the porcine adipocyte polypeptide) into an expression vector. The library is then screened for members capable of expressing a protein which binds to antiporcine-adipocyte polypeptide antibody, and which has a nucleotide sequence that is capable of encoding polypeptides that have the same amino acid sequence as the porcine adipocyte polypeptide, or fragments thereof. In this embodiment, DNA, or more preferably cDNA, is extracted and purified from a cell which is capable of expressing the porcine adipocyte polypeptide protein. The purified cDNA is fragmentized (by shearing, endonuclease digestion, etc.) to produce a pool of DNA or cDNA fragments. DNA or cDNA fragments from this pool are then cloned into an expression vector in order to produce a genomic library of expression vectors whose members each contain a unique cloned DNA or cDNA fragment.

An "expression vector" is a vector which (due to the presence of appropriate transcriptional and/or translational control sequences) is capable of expressing a DNA (or cDNA) molecule which has been cloned into the vector and of thereby producing a polypeptide or protein. Expression vectors of the present invention may be either prokaryotic or eukaryotic. Examples of suitable prokaryotic expression vectors include pASK75 (Biometra) or pET 21a-d (Novagen). Examples of suitable eukaryotic expression vectors include pcDNA3 or pRc/RSV (In Vitrogen, Inc.).

A DNA sequence encoding the porcine adipocyte polypeptide of the present invention, or its functional derivative, may be recombined with vector DNA in accordance with conventional techniques such as those disclosed by Sambrook, et al., *supra*.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression.

The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal the initiation of protein synthesis. A promoter is a double-stranded DNA or RNA molecule which is capable of binding RNA polymerase and promoting the transcription of the "operably linked" nucleic acid sequence. The promoter sequences of the present invention may be either prokaryotic, eukaryotic or viral. Strong promoters are, however, preferred. Suitable promoters are repressible, or more preferably, constitutive. Examples of suitable prokaryotic promoters include the tetracycline (TetA) promoter for pASK75 and T7lac for pET21. Examples of suitable eurkaryotic promoters include alpha actin or beta actin. Examples of suitable viral promoters include Rous sarcoma or cyotmegala.

The present invention is also directed to an antibody specific for an epitope of the porcine adipocyte polypeptide, and the use of such antibody to detect the presence of, or measure the quantity or concentration of the polypeptide, a functional derivative thereof, in a cell, a cell or tissue extract, or a biological fluid. As used herein, the term "epitope" refers to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface groupings of

molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics. An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody.

The porcine adipocyte polypeptide of the present invention, or a functional derivative thereof, preferably having at least about 8 amino acids is used as an antigen for induction of a polyclonal antibody or monoclonal antibody (mAb). As used herein, an "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one, or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), and chimeric antibodies. Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. Monoclonal antibodies are a substantially homogeneous population of antibodies to specific antigenic epitopes. MAbs may be obtained by methods known to those skilled in the art. (See, for example Kohler and Milstein, Nature 256:495-497 (1975) and USP 4,376,110; de St. Groth, S. F. et al.. J. Immunol. Methods, 35:1-21 (1980); and Hartlow, E. et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988).

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having a variable region derived from a porcine mAb and a murine

immunoglobulin constant region. Chimeric antibodies and methods for their production are known in the art (Cabilly et al, Proc. Natl. Acad. Sci. USA 81:3273-3277 (1984); Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984); Boulianne et al., Nature 312:643-646 (1984); Neuberger et al., Nature 314:268-270 (1985); Liu et al., Proc. Natl. Acad. Sci. USA 84:3439-3443 (1987); Better et al., Science 240:1041-1043 (1988)). These references are hereby incorporated by reference.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, which are capable of binding antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less nonspecific tissue binding than an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)). Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

The reaction of the antibodies and the polypeptides of the present invention are detected by immunoassay methods well known in the art (See, for example, Hartlow et al. *supra*). The antibodies, or fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the presence of cells which express the porcine adipocyte polypeptide protein. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody coupled with microscopy, flow cytometric, or fluorimetric detection.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of the porcine adipocyte polypeptide. *In situ* detection may be accomplished by removing a histological specimen from a pig, and providing a labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody

(or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the porcine adipocyte polypeptide but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Such assays for porcine adipocyte polypeptide typically comprise incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested or cultured cells containing adipogenic cells or adipocytes, in the presence of a detectably labeled antibody capable of identifying the porcine adipocyte polypeptide, and detecting the antibody by any of a number of techniques well-known in the art, such as enzyme immunoassays (EIA or ELISA) or radioimmunoassays (RIA).

The antibody molecules of the present invention may also be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support (i.e., any support capable of binding antigen or antibodies) and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

The binding activity of a given lot of antibody to the porcine adipocyte polypeptide may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Antibodies can be used in an immunoaffinity column to purify the porcine adipocyte polypeptide of the invention by a one step procedure, using methods known in the art.

According to the present invention, a pig that is susceptible to fat deposition is treated with the porcine adipocyte protein to limit such fat deposition. This treatment may be performed in conjunction with other anti-adipogenic therapies. A typical regimen for treating swine with a propensity for fat deposition comprises administration of an effective amount of the porcine adipocyte polypeptide administered over a period of time.

The porcine adipocyte polypeptide of the present invention may be administered by any means that achieve its intended purpose, preferably to alter feed intake or limit fat deposition in a subject. For example, administration may be by various parenteral routes including, but not limited to, subcutaneous, intravenous, intradermal, intramuscular, and intraperitoneal routes. Alternatively, or concurrently, administration may be by the oral route which may be accomplished by the use of genetically-altered feedstuffs, in which the porcine leptin gene has been inserted and expressed. Parenteral administration can be by bolus injection or by gradual perfusion over time such as by implant of an osmotic delivery device. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets and capsules can also be prepared according to routine methods.

It is understood that the dosage of porcine adipocyte polypeptide administered may be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art. The

total dose required for each treatment may be administered by multiple doses or in a single dose. The porcine adipocyte polypeptide of the present invention may be administered alone or in conjunction with other therapeutics directed toward the regulation of fat deposition.

In a preferred embodiment, the concentration of the porcine adipocyte polypeptide or mRNA of this invention is measured in a cell preparation, tissue extract or biological fluid of a subject as a means for determining the susceptibility or the propensity of the subject for fat deposition. The susceptibility of the subject to fat deposition is related to the level of the porcine adipocyte polypeptide or its mRNA. Additionally, restriction fragment length polymorphisms in the porcine adipocyte gene will be used to predict fat deposition potential.

Another embodiment of the invention is evaluating the efficacy of a drug or other agent, directed to the increase or decrease of feed intake by measuring the ability of the drug or agent to stimulate or suppress the production of the porcine adipocyte polypeptide, or mRNA of this invention by a cell or cell line capable of producing such polypeptides or mRNAs. Preferred cells are cells of an adipogenic cell line. The antibodies, cDNA probe or riboprobe of the present invention are useful in the method for evaluating these drugs or other agents in that they can be employed to determine the amount of the porcine adipocyte polypeptide or mRNAs using one of the above-mentioned immunoassays.

An additional embodiment of the present invention is directed to assays for measuring the susceptibility of a pig to fat deposition based on measuring in a tissue or fluid from the subject the amount of the mRNA sequences present that encode the porcine adipocyte polypeptide, or a functional derivative thereof, preferably using an RNA-DNA hybridization assay. The susceptibility to fat deposition is related to the amount of such mRNA sequences present. For such assays, the

source of the mRNA sequences is preferably a pig's adipogenic cells. The preferred technique for measuring the amount of mRNA is a hybridization assay using RNA (e.g., Ribonuclease Protection Assay) or DNA (e.g. Northern or Slot Blot Assays) of complementary base sequence.

Nucleic acid detection assays, especially hybridization assays, can be predicated on any characteristic of the nucleic acid molecule, such as its size, sequence, susceptibility to digestion by restriction endonucleases, etc. The sensitivity of such assays may be increased by altering the manner in which detection is reported or signaled to the observer. Thus, for example, assay sensitivity can be increased through the use of detectably labeled reagents. A wide variety of such labels have been used for this purpose. Kourilsky et al. (USP 4,581,333) describe the use of enzyme labels to increase sensitivity in a detection assay. Radioisotopic labels are disclosed by Falkow et al. (USP 4,358,535), and by Berninger (USP 4,446,237). Fluorescent labels (Albarella et al., EP 144914), chemical labels (Sheldon III et al., USP 4,582,789; Albarella et al., USP 4,563,417), modified bases (Miyoshi et al., EP 119448), etc. have also been used in an effort to improve the efficiency with which detection can be observed.

One method for overcoming the sensitivity limitation of nucleic acid concentration is to selectively amplify the nucleic acid whose detection is desired prior to performing the assay. Recombinant DNA methodologies capable of amplifying purified nucleic acid fragments have long been recognized. Typically, such methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by Cohen et al. (USP 4,237,224), Maniatis, T., et al., etc.

Recently, an *in vitro* enzymatic method has been described which is capable of increasing the concentration of such desired nucleic acid molecules. This method has been referred to as the "Polymerase Chain Reaction" or "PCR" (Mullis, K. et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); Erlich H. et al., EP 50, 424; EP 84,796, EP 258,017, EP 237,362; Mullis, K., EP 201,184; Mullis K. et al., USP 4,683,202; Erlich, H., USP 4,582,788; and Saiki, R. et al., USP 4,683,194). The polymerase chain reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotide probes to serve as primers for the template-dependent, polymerase mediated replication of a desired nucleic acid molecule.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE I

ISOLATION OF PORCINE LEPTIN cDNA

The putative secreted portion of porcine leptin gene product was amplified from adipose tissue mRNA using reverse transcriptase-polymerase chain reaction. Four separate cDNA synthesis reactions were carried out using 1-2 ìg of porcine adipose tissue total RNA or 1-2 ìg of poly A+ mRNA, 150 pmol of random hexamer oligonucleotides, 500 nM dNTP, 200 U of MMLV RNAse H$^-$ reverse transcriptase (Life Technologies, Inc.) in 20 ìl of the supplied buffer. The reactions were

incubated for 1 h at 37 °C and terminated by heating to 70 °C for 10 min. The leptin cDNA product was amplified by PCR using the following degenerate oligonucleotide primers with restriction site linkers for BamHI/Bsa I and EcoRI/Eco47 III, respectively:

Sense strand:

5'-GGATCCGGTCTCAGGCC GTGCC(C/T)ATCCA(A/G)AAAGTCC-3' (SEQ. ID NO. 7)

Antisense strand:

5'-GAATTCAGCGCT GCA(C/T)(C/T)CAGGGCT(G/A)A(G/C)(G/A)TC-3' (SEQ. ID NO. 8)

These oligonucleotide primers were designed from a multiple sequence alignment of the mouse and human cDNA sequences. Approximately 100 ng of adipose tissue cDNA was added as template to 50 il PCR reactions made in the manufacturers buffer with 100 pmol of each primer and 2.5 U of Taq DNA polymerase (Life Technologies, Inc.). A three stage amplification was carried out under the following conditions; Stage 1- 95 °C, 3 min; 52 °C, 1 min, 72 °C 1 min, 1 cycle; Stage 2- 94 °C, 45s; 52 °C, 45s, 72 °C, 1 min, 4 cycles; Stage 3- 94 °C, 45 s; 55 °C, 30 s, 72 °C 1 min, 28 cycles. Template cDNA from three out of four cDNA reactions produced a 466 bp product.

The PCR products were prepared for ligation into the protein expression vector pASK75 (Biometra Inc.) by complete digestion with Eco47III and partial digestion with Bsa I. The restriction enzyme digested PCR products were purified by electrophoresis in low melting point agarose and a 437 bp product was excised from the gel and ligated into the vector. The ligations were transformed in *E. coli* XL1-Blue (Stratagene Inc.) and plated on LB plates containing 50 ig/ml ampicillin for plasmid selection. Twelve *E. coli* colonies were isolated that contained the porcine leptin cDNA,

and plasmid DNA was isolated for DNA sequencing.

The nucleotide sequence of the porcine leptin gene comprising 5917 base pairs, and the amino acid translation of the leptin coding sequences are depicted in FIGS. 1A-1D (SEQ. ID NO. 1). The nucleotide sequence and the amino acid sequence of the entire porcine leptin cDNA (i.e., signal peptide and secreted proteins) comprising 501 base pairs and 167 amino acids are depicted in FIG. 2 (SEQ. ID NO. 1 and SEQ. ID NO. 2). The nucleotide sequence and the amino acid sequence of the porcine leptin cDNA corresponding to the secreted protein alone and comprising 435 base pairs and 145 amino acids are depicted in FIG. 3 (SEQ. ID NO. 3 and SEQ. ID NO. 4).

There was an 83% identity between the pig and human (SEQ. ID NO. 5) cDNA sequence and a 76% identity between the pig (SEQ. ID NO. 1) and mouse (SEQ. ID NO. 6) cDNA sequence as depicted in FIG. 4.

EXAMPLE II

ISOLATION OF mRNA CORRESPONDING TO PORCINE LEPTIN cDNA

The porcine leptin cDNA was used as a probe for detection of the full length mRNA. A northern blot containing porcine adipose and bovine adipose poly A+ mRNA as well as *ob/ob* mouse adipose total RNA was provided by Dr. M. Spurlock of Purina Mills Inc. The blot was hybridized with an [^{32}P] dCTP labeled porcine leptin cDNA in hybridization solution (HY; 0.9 M NaCl, 0.09 M sodium citrate, 0.05% ficoll, 0.05% polyvinylpyrolidone, 0.05% BSA, 0.5% SDS, 0.1% sodium pyrophosphate, 10 mM EDTA and 100 mg/ml sonicated salmon sperm DNA at 60 °C for 15 h. The blot was washed to a final stringency of 0.2X SSC (0.03M NaCl, 0.003 M sodium citrate), 0.1% SDS at 60 °C and exposed to X-ray film. A 3,090 bp leptin mRNA was detected in porcine and

bovine adipose tissue and a 3,240 bp leptin mRNA was detected on *ob/ob* mouse adipose tissue. As shown in FIG. 5, lanes 1 and 2 contain the porcine adipose poly A+mRNA, lane 3 contains the adipose total RNA from a control mouse and lanes 4 and 5 contain the adipose total RNA from an *ob/ob* mouse, and lane 6 contains the bovine adipose poly A+mRNA.

EXAMPLE III

ISOLATION OF GENOMIC DNA CLONE CORRESPONDING TO PORCINE LEPTIN

The porcine leptin cDNA was also used to screen a porcine genomic DNA library. Specifically, a porcine genomic library containing 4.64 X 10^5 recombinants was previously constructed in SuperCos 1 (Stratagene, Inc.) and screened for porcine leptin. Specifically, two sets of replica filters were prehybridized for 2 h at 60°C. Filters were hybridized overnight with [$^{-32}$P] dCTP labeled probe at 5 X 10^5 cpm per ml of hybridization solution at 65°C. Filters were sequentially washed in 2X SSC (0.3 M NaCl, 0.03 M sodium citrate), 0.5% SDS; 1X SSC, 0.5% SDS; and 0.2X SSC 0.5% SDS with each wash at 60°C for 30 min. Positive clones that showed signals on both replica filters were recovered from the agar plates and individual colonies were isolated by a second low density replica plating and hybridization step. A cosmid designated Obg-361 was isolated that hybridized to the porcine *ob* cDNA probe and had essentially the same restriction enzyme digestion pattern as found in porcine genomic DNA.

FIG. 6 illustrates the isolation of the cosmid Obg-361. Specifically, lanes 1-4 are an agarose gel containing Kb ladder molecular mass markers (lane 1), cosmid Obg-361 digested with Eco RI (lane 2) and Hind III (lane 3) and biotinylated lambda/Hind III molecular mass markers (lane 4).

Southern blot analysis of the gel in lanes 2-4 were probed with the porcine leptin cDNA indicate that the EcoRI fragments (lane 5) and the Hind III fragments (lane 6) contain leptin sequences. Lane 7 is lambda/Hind III molecular mass markers.

Porcine genomic DNA digested with BAM HI (lane 8), EcoRI (lane 9) and Hind III (lane 10) and hybridized with a Bsa I fragment (300 bp) of the porcine leptin cDNA showed equivalent bands that contain leptin sequences indicating that the porcine leptin gene was isolated in cosmid Obg-361.

The 5917 bp Hind III fragment was subcloned into Bluescript II SK+ (Stratagene, Inc.). Both strands of the sequence was determined using progressive nested deletions using Exonuclease III and Mung Bean nuclease. Sequencing reactions were carried out with Sequenase V2.0. This sequence was 5917 bp in length and contains the entire coding region in two exons (FIG. 1, SEQ. ID NO. 1). There was 78.6% nucleotide identity between the pig and human as well as 71.2% nucleotide identity between pig and mouse coding sequences. The splice junctions for the two exons were confirmed by the cDNA sequence. The cDNA sequence of the protein coding region is shown in FIG. 2 (SEQ. ID NO. 1 and SEQ. ID NO. 2). The 501 bp sequences encodes 166 amino acid residue leptin polypeptide with a predicted molecular mass of 18,334 Da.

A clone was obtained using the process described above, Obg H3-15, was deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md., 20852-1776, on July 11, 1996, and has been designated ATCC No. 97653. This microorganism was deposited under the conditions of the Budapest Treaty on the International Recognition of Deposit of Microorganisms for the purpose of Patent Procedure. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. This deposit will be maintained for a time period of 30 years from the date of deposit or 5 years after the last

request for the material, whichever is longer.

EXAMPLE IV

PURIFICATION OF THE PORCINE LEPTIN GENE PRODUCT

The polypeptide sequence encoded by the porcine leptin cDNA was synthesized and purified using the Strep-Tag system (Biometra, Inc.). The pASK plasmid contains the *omp*A leader sequence for secretion of the protein into the periplasmic space of *E. coli* as well as a ten amino acid carboxyl terminus that binds to strepavidin for affinity chromatography. Synthesis of the porcine leptin protein by *E. coli* strain XL1-Blue was induced with 200 ìg/l of anhydrotetracycline and the cells harvested after 3 h. The proteins in the periplasmic space were isolated by osmotic shock by suspending the cells in 100 mM Tris-HCl pH 8.0, 500 mM sucrose, 1 mM EDTA and 0.02% NaN_3 for 30 m at 4 °C. The cells were removed by centrifugation and the porcine leptin protein was purified from the periplasmic proteins by strepavidin affinity chromatography as depicted in FIG. 5.

Specifically, FIG. 7 shows the polyacrylamide gel electrophoresis of porcine leptin protein induction and purification in *E. coli*. Molecular mass markers are located in lane 1. Lane 2 contains total protein from XL-1 Blue and an pASK/Ob cell line before (lane 3) and after (lane 4) anhydrotetracycline induction. Affinity purified porcine leptin protein is located in lane 6.

EXAMPLE V

ANTIBODIES TO PORCINE LEPTIN PROTEIN AND THEIR USE TO DETECT PORCINE LEPTIN IN ADIPOGENIC CELLS

Polyclonal and/or monoclonal antibodies are produced with the recombinant porcine leptin protein. The techniques used for producing, screening, detecting, and/or quantifying antibodies for

porcine leptin are discussed extensively in "Antibodies: a Laboratory Manual" (Harlow et al., 1988, Cold Spring Harbor laboratory). All media or medium components, mouse or cell strains (e.g. BALB/C mouse, sp2/0 myeloma cells, JA744A.1 macrophages etc.) are commercially available.

A. Immunization of Animals

1. Rabbits:

Purified porcine leptin protein is injected into rabbits for production of polyclonal antibodies. Specifically, each rabbit receives repeated subcutaneous injections with antigen in Freund's complete adjuvant followed by at least 1 booster injection of about 200 ìg to 1 mg. When the serum titer of the immunized rabbits is sufficiently high when tested using the porcine leptin as antigen, rabbit serum is harvested as the polyclonal antiserum for porcine leptin.

2. BALB/C mice (4-week old):

Purified porcine leptin protein is injected into BALB/C mice for production of monoclonal antibodies. Specifically, each mouse is injected with about 50 ìg porcine leptin protein with Ribi 's S-TDCM adjuvants (RIBI ImmunoChem Research, Inc., Hamilton, Montana). The number of injections depends on the titer of the antibody in the serum of immunized mice as determined by ELISA using porcine leptin as the antigen. In the course of producing monoclonal antibodies against porcine leptin protein, the spleens of immunized mice are used to prepare spleenocytes. Hybridoma cells are made by fusing the spleenocytes with sp2/0 myeloma cells (treated with 8-Azaguanine containing medium) in the presence of 50% PEG-1500. Hybridoma cells are incubated in selection HAT (hypoxanthine, aminopterine, and thymidine) medium. Subsequent screening for positive

clones uses the recombinant porcine leptin as antigen in ELISA methodology. Positive clones that produce strong anti-porcine-leptin antibody are characterized for specificity, subtype, affinity, binding sites, etc.

When large quantities of purified antibody are needed, the positive clones are cultured in large scale and antibody purified from the culture supernatant, or injected into the intraperitoneal cavity of BALB/C mice for production of ascites. The latter procedure requires about 1-2x10^6 hybridoma cells per mouse, and usually takes about 7-14 days. Large quantities of antibody is then purified from ascites by ammonium sulphate precipitation and ion exchange chromatography (e.g. DEAE-Trisacryl M).

EXAMPLE VI

TOTAL RNA ISOLATION

Total RNA was extracted from subcutaneous adipose tissue according to the method reported by Chomczynski and Sacchi (1987). Tissue was homogenized in 4M guanidinium thiocyanate followed by addition of 0.1 volume of 2M sodium acetate (pH 5.0). The samples were extracted sequentially with water-saturated phenol and chloroform:isoamyl alcohol (24:1) and the aqueous fractions precipitated with isopropanol. After a second precipitation in ethanol, the RNA pellets were resuspended in 10 mM Tris, 1 mM EDTA (pH 8.0) and analyzed by spectrophotometry for quantification (A_{260}) and qualitative (A_{260}:A_{280}) determinations.

EXAMPLE VII

REVERSE TRANSCRIPTASE - POLYMERASE CHAIN REACTION

A porcine *ob* (*obese* gene) cDNA probe was amplified from adipose tissue mRNA using the reverse transcriptase-polymerase chain reaction (RT-PCR). First strand cDNA synthesis reactions were carried out using 1-2 μg of porcine adipose tissue total RNA, 150 pmol of random hexamer oligonucleotides, 500 nM dNTP, 200 U of Superscript II reverse transcriptase (LifeTechnologies, Inc., Bethesda, MD, USA) in 20 μl of the supplied buffer. The reactions were incubated for 1 h at 37 °C and terminated by heating to 70°C for 10 min. The *ob* cDNA product was amplified by PCR using the following degenerate primers with restriction site linkers for BamHI and XbaI respectively; sense strand 5'-GTGCCYATCCARAAAGTCC-3' and antisense strand 5'-GCAYYCAGGGCTRASRTC-3'. Adipose tissue cDNA was added as template to 50 μl PCR reactions made in the manufacturer's buffer with 100 pmol of each primer and 2.5 U of Taq DNA polymerase (LifeTechnologies, Inc.). A three stage amplification was carried out under the following conditions; Stage 1- 95°C, 3 min; 52°C, 1 min; 72°C, 1 min; 1 cycle; Stage 2- 94°C, 45s; 52°C, 45s; 72°C, 1 min; 4 cycles; Stage 3- 94°C, 45 s; 55°C, 30 s; 72°C 1 min; 28 cycles. The PCR products were digested with the restriction enzymes BamHI and Xba I and purified by electrophoresis in 1% NuSieve low melting point agarose (FMC Bioproducts, Rockland, ME, USA). The *ob* cDNA was ligated into Bluescript II SK+ (Stratagene Inc. LaJolla, CA, USA) and transformed into MCR DH5α (LifeTechnologies, Inc.) and plated on LB plates containing 50 μg/ml ampicillin for plasmid selection. Twelve *E. coli* colonies were isolated that contained the porcine *ob* cDNA and plasmid DNA was isolated for sequencing. Dideoxy sequencing reactions were carried

out using [^{35}S] dATP labeling with Sequenase V2.0. The sequence samples were loaded on 5% Long Ranger (FMC Bioproducts) for denaturing gel electrophoresis according to the manufacturer's recommendations.

EXAMPLE VIII

CONSTRUCTION OF THE ANTISENSE RNA

Antisense RNA may be used in vitro and in vivo to block translation of the sense RNA. A short cDNA sequence for transcription of a labeled antisense *ob* RNA was amplified by RT-PCR using the original sense strand primer and antisense GSP1 under the conditions described above. The PCR product was ligated into Bluescript II SK+ and confirmed by DNA sequencing. *In vitro* transcription with T7 RNA polymerase in the presence of [^{32}P]-UTP produces a radiolabeled 178 bp antisense RNA that was protected from T1 RNase digestion when hybridized with porcine adipose RNA. The *in vitro* transcription and RNAse protection assays were done using the Maxiscript T7 and RPA II kits (Ambion Inc., Austin, TX, USA). Specific hybridization of the antisense with the sense *ob* mRNA is adipose tissue is shown in FIG. 8. Total RNA was extracted from porcine adipose and 20 μg hybridized with the antisense riboprobe. The *obese* mRNA was protected from nuclease (RNAse) digestion when hybridized with the antisense RNA. Lanes 1-3 contained the marker, undigested antisense probe, and the digested probe, respectively. Lane 4 contained 5 μg yeast RNA, which failed to protect the antisense from digestion. Lanes 5-8 contain RNA obtained from the adipose tissue of individual pigs.

EXAMPLE IX

PRODUCTION OF THE RECOMBINANT PORCINE LEPTIN PROTEIN

The putative secreted portion of the porcine *ob* gene product, leptin, was amplified from adipose tissue mRNA and cloned into the pET24a plasmid which places an N-terminal T7 epitope tag and 6 histidine residues on the C-terminus for immobilized metal affinity chromotography. The protein has not been sequenced, but does react with the T7 epitope tag antibody, and the anti-N-terminus and α-C1 antibodies (discussed below). Aliquots of the recombinant protein were assayed for activity in an in vitro lipolysis assay with isolated porcine adipocytes. Several laboratories have reported that leptin stimulates lipolysis in rodent adipocytes and counters the anti-lipolytic action of insulin (Müller et al. ,1997; Fruhbeck et al., 1998; Wang et al., 1999). We hypothesized that this is also true of porcine adipocytes, and the preliminary data support this hypothesis. We compared two concentrations of recombinant human leptin (25 and 150 ng per mL) and a single concentration of recombinant porcine leptin (25 ng per mL) with submaximally-activating concentrations of isoproterenol as a positive control. Isoproterenol is a potent ß-adrenoceptor agonist that stimulates lipolysis in adipocytes. Adipocytes were isolated by collagenase digestion from a pig weighing about 45 kg. The adipocytes were diluted to an approximate 20% suspension in DMEM + 3% serum albumin and incubated for 6 hours at 37 C. Recombinant human leptin (L) or recombinant porcine leptin (rPL) was added at 25 or 150 μg per mL. Isoproterenol (ISO) was added at 10^{-8} and 10^{-7} M to confirm the validity of the cell preparation. Each treatment was replicated 3 times. The glycerol concentration of the incubation medium was used as the index of lipolysis. Means (gray) ± SE (hatched bars). All treatments

resulted in an increase ($P < .04$) in lipolysis vs. the basal treatment. As shown in FIG. 9, our results indicated a near doubling ($P < .04$) of basal lipolysis by leptin, irrespective of source, and it was apparent that 25 ng per mL was adequate to stimulate lipolysis in porcine adipocytes.

EXAMPLE X

ANTIBODIES TO PORCINE LEPTIN

Rabbit polyclonal antibodies were initially made to synthetic peptides derived from the N-terminus of the secreted portion of the protein (WRVQDDTKTLIKTIVTRISD) as a map peptide and the C-terminus (C1 peptide, LQGALQDMLRQLDLSPGC) for conjugation to keyhole limpet hemocyanin. Both peptides produced antibodies in rabbits that cross-react with the recombinant pig leptin produced using the pET24a *E. coli* expression system. The C-terminus peptide produced a very high titer antiserum (α-C1) that contains significant activity at 1:50,000 dilution. The α-C1 antiserum was particularly rigorous and retained activity in detergent conditions up to 1% SDS. This antibody is satisfactory for Western blot detection of the recombinant human leptin and the recombinant porcine leptin (FIG. 10). This antibody is used for Western blot detection of the recombinant human leptin (lanes 1-4, 14.8 kDa) obtained from Eli Lilly & Co., and the recombinant porcine leptin (lanes5-8, 16 kDa). Lanes 7 and 8 reveal a dimer at approximately 33 kDa). Lanes 7 and 8 reveal a dimer at approximately 33 kDa. The data presented are a concentration curve (.05 - 1 ìg) of each protein.

Additionally, antiserum from rabbits immunized with the recombinant (T7-tagged) porcine leptin was affinity purified and used to immunoprecipitate leptin from biological fluids and adipose tissue extracts as shown FIG. 11. Specifically, antiserum from rabbits immunized with recombinant

(T7-tagged) porcine leptin was affinity purified and pre-immune IgG purified from serum by protein A chromatography. The affinity purified anti-T7-leptin antibodies (α-T7-leptin) immuno-precipitated a protein (about 16 kDa) from pig serum, cerebral spinal fluid (CSF) and adipose tissue extracts that was not precipitated by the pre-immune IgG. The α-C1 antibody was used to detect the precipated leptin on the Western blot. Immune serum (I) yielded strong signals in serum, CSF, and adipose extracts, whereas preimmune serum (P) did not. These antibodies and the recombinant protein are being used to construct a porcine leptin radioimmuno assay (RIA).

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation. While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.